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Article

Microbial Keratinase- An Essential Enzyme for Production of Feather hydrolysate in Normal Clay Pot

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ABSTRACT

The widespread production of keratin-rich byproducts as agricultural and industrial waste emphasizes the importance of their proper utilization. Many studies believe that chemical approaches for treating keratin waste are hazardous to the environment because they emit secondary pollutants. In contrast, microbial degradation of keratin waste is a new, environmentally acceptable alternative that provides two benefits: the treatment of refractory keratin pollutants and the generation of the economically valuable enzyme keratinase. It also goes over the various uses of microbial keratinases in waste management, animal feed, detergent, fertilizer manufacturing, and industries including leather, cosmetics, and pharmaceuticals. Here we used feather and poultry litter to produce feather hydrolysate with the help of *Bacillus wiedmannii* SAB 10. In this investigation we observed total amino acid concentration- 707 µg/ml, Total protein- 4.25 mg/ml, Oligopeptides- 6.98 mg/ml, Total Thiol- 84.3 µg/ml, IAA content - 42.34 µg/ml, Ammonia production - 13.31 µg/ml. utilizing clay pots for fermentation is an environmental friendly, cost effective and sustainable approach, as it is easily available possess inherent anti microbial properties that prevent contamination and spoilage, can be reused multiple times.

Introduction

Keratin is a protein that forms strong fibers and is an essential component of both epidermal and skeletal tissues. Its insoluble nature and resistance to enzymatic digestion by enzymes such as trypsin, pepsin, and papain are the result of significant cross-linking assisted by disulphide bonds, hydrogen bonds, and hydrophobic interactions (Cohlberg, 1993; Steinert, 1993). Keratinases are prominent proteolytic enzymes capable of degrading keratin present in chicken feathers, animal wool, and hair, all of which are commonly viewed as chronic waste. Keratin makes up about 90 % of the weight of feathers. Feather keratin is high in glycine, alanine, serine, cysteine, and valine keratinase enzymes are primarily members of the serine Endo protease family, which is distinguished by the presence of serine residues in their active sites, which aid in the breakage of peptide bonds inside polypeptide chains. Keratinases have exceptional action on insoluble keratin, making them critical for breaking down feathers, hair, wool, collagen, and other keratin-rich materials that frequently contribute to solid waste problems .These enzymes are now used to maintain and clean sewage systems, and they are popular for their prospective applications in different industries, including food, textiles, medicine, and cosmetics. Notably, keratinases are well known for their usage in dermatological therapies, such as treating acne and psoriasis and assisting in the removal of calluses in medical settings. Furthermore, they show promise as a vaccine component for dermatophytosis treatment (Gupta and Ramnani, 2006). Keratinases are being used in the leather processing industry as an environmentally benign alternative to old chemical procedures. Conventional procedures frequently result in considerable discharges of sodium sulphide and other detrimental chemical to aquatic bodies, making the leather industry a major contributor to water pollution. Keratinases provide a long-term option for reducing environmental effect by replacing these technologies. (Verma et al, 2011). Since the discovery of keratinases' water solubility and proteolytic characteristics, several applications have emerged. One of the most notable recent developments is the bioconversion of poultry manure into bioenergy. The chicken industry generates a large amount of feather waste, which is an excellent source of keratin. This keratin can also be used to make slow-release nitrogen fertilizers, biodegradable films, peptides, and amino acids (Bressollier et al, 1999). Recent advances in keratinase production have sparked widespread interest, owing to the enzyme's significance in controlling keratin-rich waste. For industrial applications, there is an increasing demand for cost-effective keratinase synthesis in huge quantities. Researchers have identified several microbiotas in that can produce large amounts of this enzyme. Amino acids are suitable for manufacturing animal feeds, feather meals, and bio-fertilizers. Unfortunately, inadequate waste management results in their disposal in huge amounts, causing environmental concerns (Paul et al., 2013). Traditionally, feathers were either burnt or turned into low-quality animal feed using high-pressure or high-temperature treatments. However, these technologies have substantial downsides, including the loss of essential amino acids such as tryptophan, methionine, and lysine, which decreases the protein content, digestibility, and nutritional value of the feed. Furthermore, these processes are energy-intensive and release dangerous greenhouse gases, harming both human health and the environment (Fang et al., 2013a; Sharma and Devi, 2018). To address these concerns, a new and ecologically benign biocatalyst for keratin degradation is urgently required. Microbial keratinases, which are extraordinary enzymes produced by a variety of microorganisms actinomycetes, yeasts, and fungi, have showed considerable potential in this area. These enzymes can effectively convert keratin-containing wastes into important products such as amino acids, animal feed, adhesives, biodegradable films, and fertilizers by using diverse agro-industrial substrates such as chicken feathers, wool, hair, and nails (Nagal and Jain, 2010). The global surge in meat consumption over the years has resulted in a huge increase in waste generated by the meat industry. This comprises viscera, skins, meat trimmings, bones, blood, and epidermal attachments (Meruane and Rojas, 2012).In present scenario for the population growth in increasing demand for food has resulted in greater use of synthetic fertilizers, particularly nitrogen (N), which is an essential component for plant growth. Fertilizers are intended to improve plant growth and production, however over use of chemical fertilizers can cause soil pollution, loss of soil fertility, and water contamination via leaching. This can also have a negative impact on plants, such as decreased productivity and root burn. Finding alternatives to synthetic fertilizers has become crucial. Due to the low recyclability, poultry sector products such as chicken feathers and poultry litter frequently contribute to environmental pollution, as the majority of this waste is either disposed in wrong way (Gupta and Sharma, 2023). In our investigation we used two wasted products (feather and Poultry litter) emitted from poultry processing industries and main focused of this study was minimize the control of environmental pollution as well as reduce the use of synthetic fertilizers to the agricultural land. Feather hydrolysate from microbial degradation of feather are a valuable source of peptides, soluble proteins, and amino acids that enhance microbial activity in the rhizosphere, hence improving plant nutrient uptake and utilization. These hydrolysates also increase soil qualities like water retention, mineral content, and (C/N) ratio. Animal feather is a good nitrogen source and has the potential to be in biofertilizer in agriculture, reducing dependency on traditional inorganic fertilizers (Gupta and Sharma, 2023). Utilizing clay pots for fermentation is an environmentally friendly, cost effective and sustainable approach, as it is easily available possess inherent anti-microbial properties that prevent contamination and spoilage, can be reused multiple times.

Methods

Collection of raw materials

Poultry feathers and litter were collected from Debra, Paschim Medinipur, West Bengal, India, while the clay pot used for the process was procured from a local market at Debra. All reagents required for the observation were obtained from local suppliers at of Debra, Paschim Medinipur, West Bengal, India.

The keratinolytic bacterium Bacillus wiedmanni SAB 10 (Sahoo et al, 2022) utilized in this study was previously identified from a poultry waste disposal site of Debra, Paschim Medinipur, West Bengal, India.

Production of feather hydrolysate in clay pot

The production of feather hydrolysate begins with sterilizing a clay pot containing rice straw, addition of sterilized liquid poultry litter, stalks, and feathers. The fermentation process is then initiated by introducing the keratinolytic bacterium *Bacillus wiedmannii* SAB 10 previously isolated for its efficiency in keratin degradation. Over a 48-hour period, various parameters are monitored, including total amino acid concentration by ninhydrin Method (Moore and Stein. 1954), total protein content by Lowry method (1951), Oligopeptides (Gerard et al, 2004), The determination of thiol content was measured by the method described by Sullivan et al. (1942), indole-3-acetic acid (IAA) content by Salkowski reagent (Malik and Sindhu, 2011), and ammonia production (Geetha 2014), to evaluate the effectiveness of the process. The parameters (Concentration of Feather, concentration of poultry Litter, pH, Temperature, Time and inoculums value) that influences the feather degradation in static fermentation process was previously optimized in OVAT (one Variable At a Time) system (Sahoo et al, 2023). previously scientist investigate that normal clay pot offer a great fermentation container in comparison to the other container (Kebede et al, 2007). All the process describes in Fig 1.

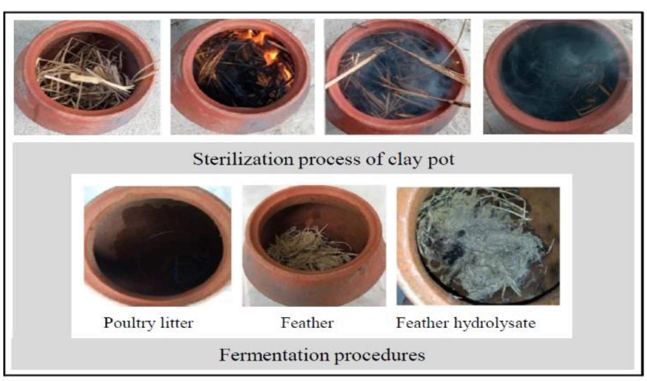

Figure 1: Fertilizer Stability Analysis

An essential aspect of evaluating any fertilizer is determining its effectiveness over time on plants or other applications. In this study, the performance of the fertilizer was assessed by analyzing and monitoring the amino acid content of the feather hydrolysate.

Results and Discussion

After 48 hours of incubation period all the following parameters were measured. Total amino acid concentration- 707 µg/ml, Total protein- 4.25 mg/ml, Oligopeptides- 6.98 mg/ml, Total Thiol- 84.3 µg/ml, IAA content- 42.34 µg/ml, Ammonia production - 13. 31 µg/ml. After 90 days, it was observed that the colour of feather hydrolysate shifted to a darker, blackish and its concentration was gradually decreased. When feather hydrolysate away from direct sunlight it showed considerable storage stability at room temperature without any dilution. But exposure to direct sunlight reduced the activity of amino acids concentration for oxidative effects on the thioester groups of diverse amino acids present in the solution (Han et al, 2018). This study emphasizes the liquid fertilizer's good stability under temperature flactuation. The amino acid profile of the feather hydrolysate remained unchanged after 30 days at 4°C. Previously several scientists use the feather to produce feather hydrolysate but our investigation focuses on two major wasted products that is easy to access. Different bacillus strain produces feather protein hydrolysate but our strain comparatively produces more protein than other (Mazotto et al, 2011). All the details were given in Table 1.

Table 1: Feather hydrolysate stability analysis

Day Interval	Amino acid content (µg/ml)
1 days	707
7 days	698
10 days	691
15 days	684
21 days	675
30 days	663
40 days	651
45 days	643
50 days	638
55 days	629
60 days	621
65 days	617
70 days	608
75 days	598
80 days	588
85 days	580
90 days	571

The widespread production of keratin-rich byproducts as poultry farming and industrial waste emphasizes the importance of their proper utilization. Many studies believe that chemical approaches for treating keratin waste are hazardous to the environment because they emit secondary pollutants. In contrast, microbial degradation of keratin waste is a new, environmentally acceptable alternative that provides two benefits: the treatment of refractory keratin pollutants and the generation of the economically valuable enzyme keratinase. This investigates the ability of several bacterial and fungal species to produce keratinase using keratin-rich wastes as growth substrates. It also goes over the various uses of microbial keratinases in waste management, animal feed, detergent and fertilizer manufacturing, and industries including leather, cosmetics, and pharmaceuticals.

Conclusion

The poultry processing industry is quickly expanding, generating millions of tones of feather waste annually. Many conventional chemical treatments for handling keratin waste are not suggested by researchers since they emit extra contaminants. Keratinase, an enzyme produced by diverse micro biota such as bacterium and fungi, is a viable solution for keratin waste treatment. Beyond its role in feather waste management, keratinases great resistance to organic solvents and extreme temperatures make it a promising candidate for usage in a variety of industrial applications. since a result, using keratinolytic microorganisms to treat keratin-rich waste is highly recommended, since it offers both environmental and industrial benefits. Chicken feather waste hydrolysate is ideal for organic farming since they maintain soil microorganisms while also enhancing the ecology. Our finding focuses on the importance of biofertilizer obtained from chicken feather waste, other biological residues, as well as the prospective applications of keratin and feather hydrolysate in promoting sustainable agricultural practices. Our findings benefit farmers by proving that waste products and widely available clay pots are both inexpensive and accessible options.

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